

Biosynthesis of silver nanoparticles using fresh extracts of *Tridax procumbens* Linn

Himakshi Bhati–Kushwaha & CP Malik*

School of Life Sciences, Jaipur National University, Jagatpura, Jaipur, India 302 017

Received 12 March 2013; revised 18 November 2013

A simple and eco-friendly method for the synthesis of biogenic nanoparticles (NP's) using an aqueous solution of *T. procumbens* fresh plant extract (leaf and stem) as a bioreductant is reported. The prepared biogenic nanoparticles were well characterized using U.V. visible spectroscopy, scanning electron microscopy, X-ray diffraction and Fourier-transform infrared spectroscopy. The particles were confirmed to be elemental crystal by X-ray diffraction. The potential applications of biosynthesized nanoparticles as antimicrobial (antibacterial and antifungal) against pathogens *Escherichia coli*, *Vibrio cholerae*, *Aspergillus niger* and *Aspergillus flavus* were demonstrated.

Keywords: Antimicrobial, Biosynthetic approach, FTIR, Nanoparticles, *Tridax procumbens*

In the past few decades synthesis of nanoparticles (NP's) from plant sources has proved to be an effective and alternate method for the novel production of nanoparticles. Synthesis of gold and silver nanoparticles can be achieved through various chemical and physical methods including the chemical reduction of silver ions in aqueous solutions, with or without stabilizing agents, thermal decomposition in inorganic solvent, chemical and photo reduction. But the limitations faced by these methods leads to an important area in nanotechnology dealing with the synthesis of nanoparticles which has encountered immense progress due to innumerable applications in recent decades¹, and therefore has witnessed as an advanced field in the methods of nanomaterial fabrication and their utilization for physicochemical and optoelectronic properties². Generally, metal nanoparticles are synthesized through various methods including chemical and mechanical methods, electrochemical techniques, and photochemical reactions in reverse micelles. Most of these methods involve toxic chemicals and are non eco-friendly. Alternatively, synthesis of nanoparticles through biological method is rapid, eco-friendly and economical³. Biosynthesis of biogenic nanomaterials and their characterization is a promising field of nanotechnology using bottom-up approach and is of

vital importance in medicinal and technological aspects. Moreover, this process of biological synthesis is of great success due to metal tolerance of these entities⁴. Reports are there on the extra cellular biosynthesis of Ag-NPs using microbes and plants and pure compounds from plants⁴⁻⁸.

Tridax procumbens Linn. (Fam: Asteraceae), abounds in alkaloids, carotenoids, flavonoids (catechins and flavones) and tannins. It is also rich in sodium, potassium and calcium⁹. The plant is used to treat various ailments, such as bronchial catarrh, dysentery, diarrhoea, preventing hair loss, and to check haemorrhage from cuts^{10,11}. Its pharmacological studies have revealed some important medicinal properties like antiinflammatory, hepatoprotective, wound-healing, immunomodulatory, antimicrobial, antiseptic, hypotensive and bradycardiac effects¹²⁻¹⁴. Plants as being a safer source of nanoparticles synthesis, *Tridax procumbens* was selected for their variable phytoconstituents and especially having high amount of ketones, phenols, alkanes, amine and lactones. All the available reports on *T. procumbens* phytoconstituents and pharmacological properties make this plant more acceptable for the present study.

In the present study, the mechanistic approach of biosynthesis of AgNPs using *Tridax procumbens* leaf and stem extract (fresh) is described. The antimicrobial (antibacterial and antifungal) activities of synthesized AgNPs against *Escherichia coli* and *Vibrio cholerae* and fungal culture of *Aspergillus niger* and *A. flavus* by disc diffusion and food poisoning method have also been tested.

*Correspondent author
Telephone: 0141-3080601-605
Fax: 0141-2752418
E-mail: cpm_malik@yahoo.com

Materials and Methods

Reagents—All the chemicals and reagents used in this study were of analytical grade. Silver nitrate was obtained from Sigma-Aldrich Chemicals. All glassware was washed in dilute HNO₃ acid and rinsed thoroughly with double-distilled water prior to use and dried in a hot air oven. pH was adjusted to the required value with 0.1 M NaOH or 0.1 M H₂SO₄.

Preparation of plant extracts—The leaves and stem of *T. procumbens* (Fam; Asteraceae), collected from JNU, Jaipur Campus were used. They were thoroughly washed with tap water to remove soil, dirt, etc. and finally with double-distilled water. To prepare the extract 25 g of leaves and stem were finely cut and were boiled in 100 mL of triple deionized water for 15 min, in a 250 mL Erlenmeyer flask individually and the solution were decanted, then filtered using Whatman's Filter Paper no.1 to obtain plant extract of definite concentrations.

Development of AgNPs—Leaf extract (10 mL) was added to 100 mL of 5 mM and 10 mM aqueous silver nitrate solution (1:9 ratio) at room temperature. The formation of AgNPs was indicated by the development of yellowish-dark brown colour. In the variation of pH, first the pH of the extract was adjusted before adding silver nitrate solution. The incubation was done for 5 h. The observations were made after each hour, to encompass a change in colour from light greenish to dark brownish. After each hour small amount of the reaction mixture was centrifuged at 18,000 rpm for 25 min and the supernatant was collected and pellet was stored at 4 °C. Similar steps were preformed with stem extract.

Characterization of AgNPs—For characterization the collected samples were first converted into powder using lyophilisation process. Prior to lyophilizing, samples were freeze dried in the deep freezer at -4 °C to form slants in the tubes. The tubes were attached in the lyophilizer machine ScanVac. The tubes were connected when the temperature became -50–52 °C. The machine was kept in operation with the slants. The latter were retained when the frozen material got shed off from the walls converting into powdered form.

UV-Vis spectral analysis was performed on a Genesys 10 UV spectrophotometer and the absorbance of the individual sample was recorded at 300–600 nm. EVO-50 INCA Penta FETx 3 SEM machine was used to characterize mean particle size as well as their morphology. The lyophilized samples

of BNP's solution were ultra-sonicated with distilled water, small drop of this sample was placed on carbon stubs and allowed to dry. The machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20 kV. The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique using ISO-DEBYEFLEX 2002 model and using Cu K α radiation. The generator voltage and current were set at 35 KV and 25 mA respectively. Ag samples were scanned in the 2 θ ranges 0-60 in continuous scan mode. The scan rate was 0.04 sec⁻¹. Phases present in the sample has been identified with the search match facility available with Philips X'pert high score software. The crystallite size of the powders was determined from X-ray line broadening using the Scherrer's equation:

$$D = 0.94 \lambda / \beta \cos \theta$$

where, D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle.

FTIR spectrum was recorded using KBr pellet formation using about 2 mg of the sample powder. This mixture was then subjected to dye applying the hydrolic pressure of about 1.5 bar for few seconds. The pellet was carefully taken on the sample holder and subjected to FTIR analysis. For this BRUKER-VERTEX-70 Machine was used. The software used for the analysis of the sample was OPUS.

Antibacterial assay—Antibacterial assay of synthesized nanoparticles was performed by food poisoning and agar disc diffusion methods on pathogens *Escherichia coli* and *Vibrio cholerae* using antibiotics (tetracycline, penicillin and streptomycin) as control. Freshly prepared inoculums of the selected strains were spread on their respective medium (100 μ L) containing Petri plates. Inoculated Petri plates were incubated at 37 °C for 48 h and observation was recorded.

Antifungal assay—The antifungal activity of Ag-NPs was evaluated against the pathogenic strains *Aspergillus niger* and *Aspergillus flavus* by food poisoning method. During the process individual fungal strains were point inoculate and incubated at 28 °C for 72 h. The diameter of mycelia colony developing on the nanoparticles containing PDA plates was compared with the diameter of colony obtained on control plates (devoid of the synthesized

nanoparticles). The inhibition of growth (in %) was calculated by :

$$\text{Inhibition (\%)} = (C-E) \times 100/C$$

where C is diameter of the mycelia colony in control and E is diameter of the mycelia colony with the synthesized nanoparticles.

Results

Formation of nanoparticles by reduction of silver nitrate during its exposure to the plant extracts can be easily monitored by the change in colour of the reaction mixture from greenish to dark brown, clearly indicating the synthesis of the nanoparticles and confirmed by the surface plasmon resonance and the oscillation of free electron²⁴ (Fig. 1 a and b). The reduction of silver was analyzed by using the UV-Vis Spectrophotometer. Absorption spectra of Ag-NPs formed in the reaction mixture had absorbance peak ranging from 410-430 nm, and broadening of peak indicated that the particles are polydispersed. The frequency and width of the surface plasmon

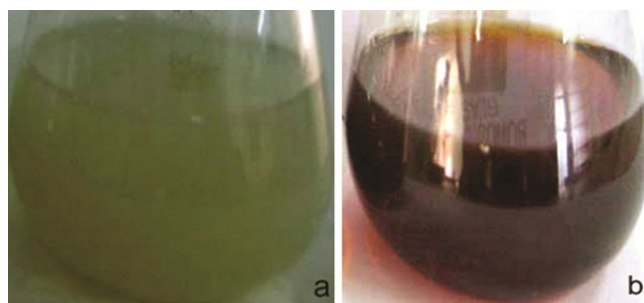


Fig. 1—*T. procumbens* (a) and (b).change in colour of the leaf reaction mixture (LRM) (5 mM of AgNO₃)

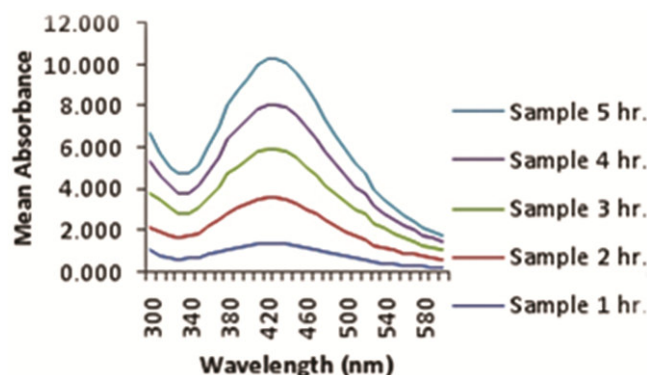


Fig. 2—Showing the peak values of the synthesized TNP (fresh leaves) using AgNO₃ (5 mM and 10 mM)

absorption depended on the size and shape of the metal nanoparticles as well as surrounding medium etc. (Fig. 2).

The biosynthesised silver nanostructure by using extract of *T. procumbens* was further confirmed by the characteristic peaks observed in the XRD image at 2θ with min and max range at 19.4-23.33°. Several Bragg's reflections pointed towards crystal structure of silver. Moreover, the XRD pattern suggested that the Ag-NPs are crystalline in nature (Fig. 3).

The SEM image (Fig. 4) showed high density Ag-NPs synthesized by the *T. procumbens* additionally authenticated the development of silver nanostructures. The SEM micrographs of nanoparticle obtained in the filtrate showed that Ag-NPs are spherical in shape and were poly dispersed without conglomeration in solution. The silver nanoparticles synthesized presently were scanned using SEM and XRD to reveal their average mean size ranging from 13.51–17.24 nm and seems to be spherical.

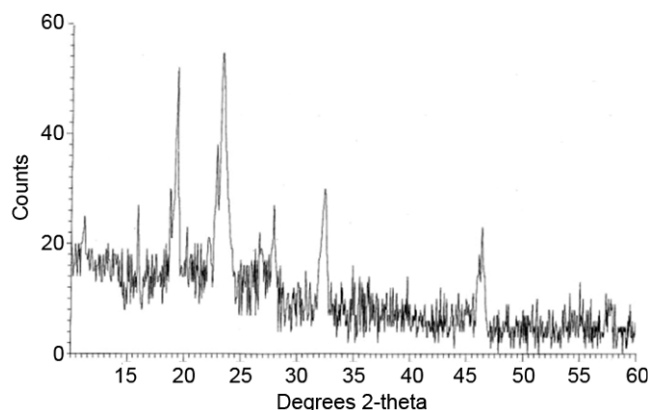


Fig. 3—Graph denoting the XRD Analysis of the TNP (fresh leaf)

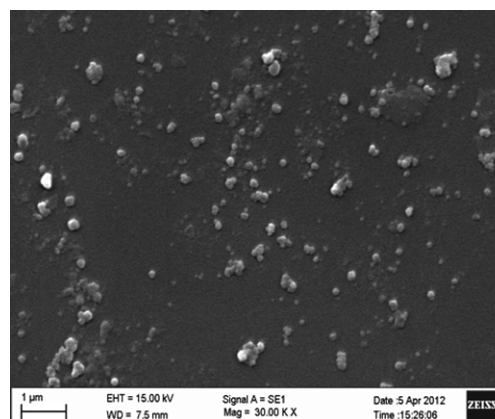


Fig. 4—Signifies the SEM images of TNP (fresh leaf)

The resulting nanoparticles were characterized through FTIR analysis. Based on absorbance bands different resultant groups were identified (Table 1) which was assigned to the stretching vibrations of primary and secondary amines, respectively (Fig. 5).

The evaluation of antibiotic resistant pathogenic fungi should encourage the search for effective antifungal agents from alternative diverse sources. Studies are available to show the antimicrobial effects of nano-Ag. However, only limited literatures supports the effects of Ag-NPs against fungal pathogens. Further the nanoparticles synthesis by

green route by using *T. procumbens* extract was found highly active against tested fungal species at a concentration of 50 ppm of synthesized Ag nanoparticles. The results showed higher antifungal activity against *Aspergillus* sp (Fig. 6 and Table 2). The present investigation demonstrates a simple, rapid and economical route to synthesize Ag-NPs from plants. The zone of inhibition convincingly showed that the fungal strains tested were susceptible to silver nanoparticles. Further, the present study proved that the biogenic nanoparticles synthesized appear to be promising and effective antibacterial agents at very low concentrations (30 and 40 ppm), against the bacterial strains selected for the present study (Figs 7-9 and Tables 3-5). The evaluation of the activity of synthesized biogenic nanoparticles was also compared for the first time with three different extracts of plant i.e. aqueous, ethanolic and methanolic extracts of leaf and stem individually and their results are shown in Figures 10 and 11 and their calculative analysis is represented in Table 6.

Discussion

The synthesis of nanoparticles is attracting added attention because of their usually enhanced physiological properties and biological activities as compared to the bulk parent material¹⁶. Of the three routes, biological synthesis is preferred for being cost effective and the ability to maintain the homogeneity and stability of the synthesized nanoparticles.

The unarguable property of silver nanoparticles of being strongly antibacterial makes it more favourable

Table 1—Results of FTIR analysis of the synthesized nanoparticles from fresh extract (leaf and stem) of *T. procumbens*

Corresponding wave number (cm ⁻¹)	Type of peak	Resultant group
3400-3500	Broad	-OH stretching of alcohols and phenols
2900	Medium	Aldehyde stretching of alkanes and primary amines
1640-1550	Medium	N-H stretching of primary and secondary amides
1400-1500	Medium	Primary amines and alkanes
1300-1400	Medium	Ketones (aromatic), aldehyde and carboxylic group
1242-1343	Medium	C-N stretching of vibration of amines or C-H stretching of alcohols, ethers, carboxylic acid, ester and anhydrides
1114-1343	Strong	Highly symmetrical alcohols, phenols and lactones
800-850	Medium	Aliphatic amines, alkanes

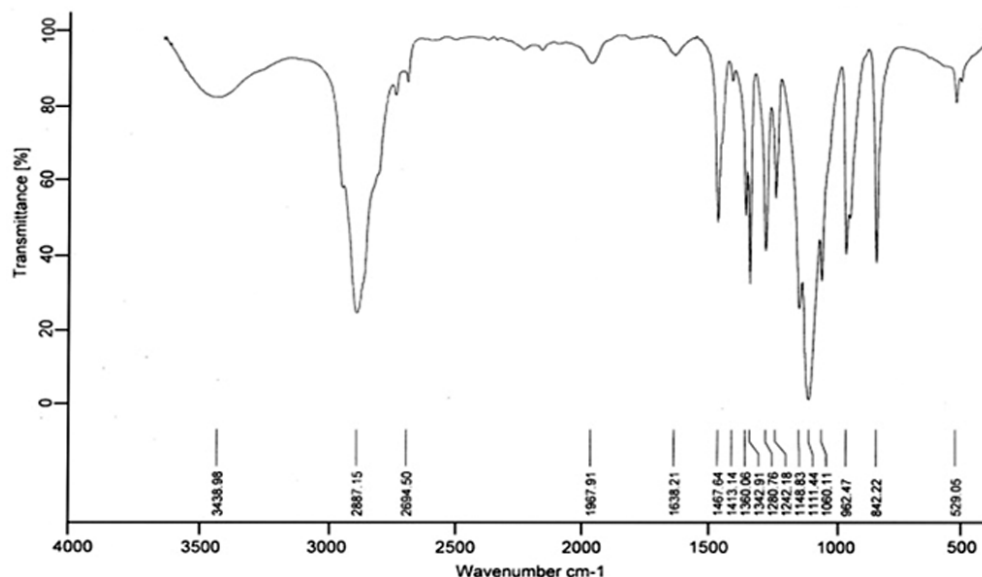


Fig. 5—Graph showing the FTIR analysis of the TNP (fresh leaf)

for the use in medical devices and supplies such as wound dressings, scaffolds, skin donation, recipient sites, and sterilized materials in hospitals, medical catheters, contraceptive devices, surgical instruments, bone prostheses, artificial teeth and bone coating.

Biosynthesis of nanoparticles has received considerable attention due to the need to develop environmentally benign technologies in material synthesis^{17,18}. Biological synthesis comprises utilization of various plant resources including microorganism, enzymes¹⁹, plant and/or plant extract and is eco-friendly. Moreover, biological system (s) tends to provide a number of metal or metal containing particles in the nanometer range.

Table 2—Antifungal test in the form of % inhibition

Organism used	Disease caused	Sample used	BNP's conc. (ppm)	Growth inhibition (%)
<i>A. niger</i>	<i>Aspergillosis, Otomycosis</i>	BNP		
		<i>T. procumbens</i> FL	50	85
		<i>T. procumbens</i> FS	50	80
<i>A. flavus</i>	Chronic granulomatous, sinusitis, keratitis, cutaneous aspergillosis	<i>T. procumbens</i> FL	50	60
		<i>T. procumbens</i> FS	50	60

Interestingly, both unicellular and multicellular organisms are reported to produce inorganic materials either intra-or extra-cellularly. Further it does not utilize any harmful chemical protocols.

Due to splitting of AgNO_3 into Ag^+ and NO_3^- change in colour of the reaction mixture was observed, with progressive time. Apparently the metabolites in the stem and leaf extract acted as e^- donor and reduce Ag^+ ions into Ag. Consequently, the formation of nanoparticles was indicated by brown colour of the

Table 3—Food poisoning method using *E. coli* and *V. cholerae* as pathogens

Disease used	Source of BNP's	Con. BNP's (ppm)	Control	Result
<i>E. coli</i>				
<i>Cholecystitis, bactremia, cholangitis, diarrhoea</i>	<i>T. procumbens</i> Fresh Leaf	30	Appearance of green metallic colonies	No growth was observed
	Fresh Stem	30	"	"
<i>V. cholerae</i>				
<i>Cholerae fever</i>	Fresh Leaf	40	Appearance of yellow colonies	No growth was observed
	Fresh Stem	40	"	"

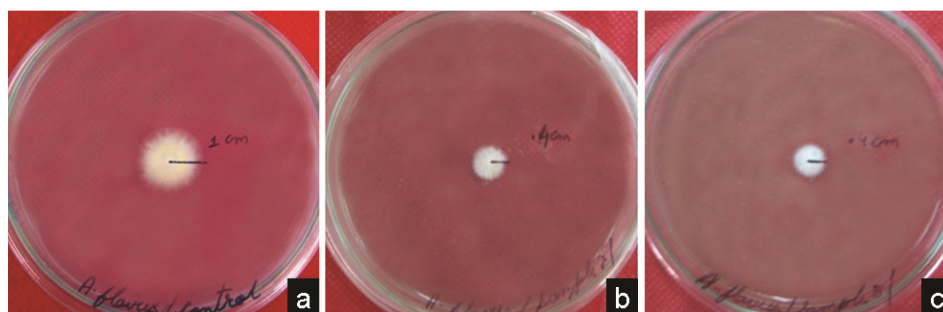


Fig. 6—Showing the *A. flavus* mycelia growth in control with no silver nanoparticle sample (a), growth of fungal mycelia when leaf (b) and stem (c) extract derived nanoparticle respectively



Fig. 7—Expressing the results of Food Poisoning Method Using *E. coli* bacteria, where CONTROL (a) is devoid of BNP's and others having BNP's derived from leaf (b) and stem (c) incorporated in EMB media (TNP- *Tridax* nanoparticles; FL- fresh leaf; FS- fresh stem)

aqueous solution following the excitation of surface plasmon vibrations.

The present findings substantiate the data from *Capsicum annum*⁴, *Aloe vera* extracts²⁰, *Citrullus colocynthis*²¹ and *Boswellia ovalifoliolata*²² except that in the present study formation of nanoparticles was accomplished at 5 and 10 mM of the aqueous solution.

Extracellular synthesis of nanoparticles was opted in the present study as this method offers great advantage over an intracellular process of synthesis, since the nanoparticles formed inside the biomass would have required additional step of processing for release of the nanoparticles from the biomass by ultrasound treatment or by reaction with suitable detergents.

The reduction of silver ions during the incubation period is generally deciphered through UV-visible spectroscopy and this period is variable ranging from few minutes to several hours. Further characterization of synthesized biogenic nanoparticles is ascertained using SEM and XRD^{23,24}. In the present study all the above mentioned techniques were used except TEM to characterize the synthesized biogenic nanoparticles. Though, biogenic nanoparticles from the powder extract of *Tridax* plant organs (leaf and stem) was already reported where the synthesized nanoparticles were of size ranging from 25.96 and 35.44 nm²⁵,

which was much smaller as compared to the nanoparticles synthesized from the same by Dhananlakshmi and Rajendran²⁶. On the other hand no XRD analysis was performed by them making their results controversial as one cannot predict the exact size of the synthesized nanoparticles without XRD analysis.

During UV-visible analysis most of the absorbance peaks obtained with different sample used were located within a range of 420 nm which coincided with the results obtained with the extract of mangrove plant (leaf bud) with a absorbance peak at 426 nm²⁷. These peaks were obtained as in metal nanoparticles,

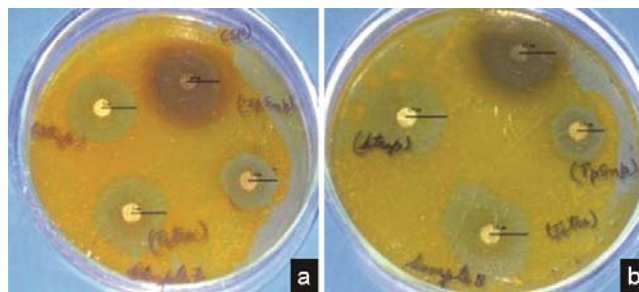


Fig. 9—Expressing the results of Agar Disc Diffusion Method Using *V. cholerae* bacterial plates incorporated with leaf (a) and stem (b) derived nanoparticles, where tetracycline and streptomycin are used as control in TCBS media (TNP- *Tridax* nanoparticles; FL- fresh leaf; FS- fresh stem)

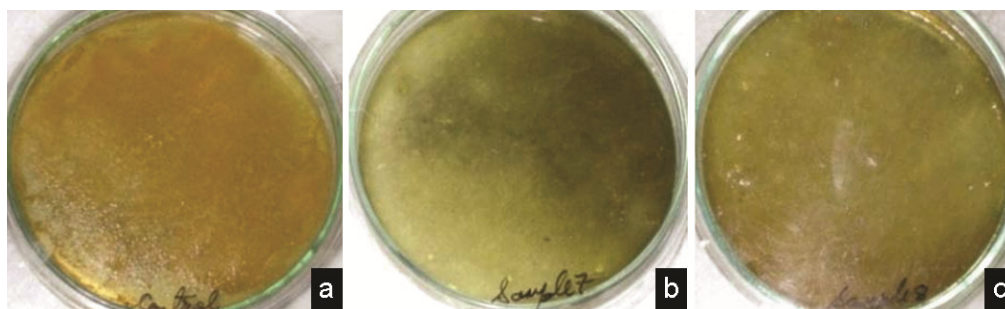


Fig. 8—Expressing the results of Food Poisoning Method Using *V. cholerae* bacteria, where CONTROL (a) is devoid of BNP's and others having BNP's derived from leaf (b) and stem (c) incorporated in TCBS media (TNP- *Tridax* nanoparticles; FL- fresh leaf; FS- fresh stem)

Table 4—Agar Disc diffusion method using *E. coli*

[Values are mean±SE of 4 replicates]

Disease caused	Sample used	Conc. BNP's (ppm)	Result (Zone of inhibition diameter mm)			
			Control			BNP's
<i>Cholecystitis, bactremia, cholengitis, diarrhoea</i>	<i>T.procumbens</i>		Tetra.	Pen.	Strep.	
	Fresh leaf	30	NSR	18±1	10±0.8	9.5±0.9
	Fresh stem	30	11±0.7	14.5±0.9	21±3.8	8

Tetra.=Tetracycline; Pen.=Penicillin; Strep.=Streptomycin and NSR=No Significant Results;
BNP's=Biogenic nanoparticles; TNP= *Tridax* nanoparticles

conduction band and valence bands lie very close to each other and through these electrons are capable of making free movement. These free electrons give rise to Surface Plasmon Resonance (SPR) absorption band. SPR results due to the collective oscillations of electrons of synthesized nanoparticles in resonance with light waves.

Once the nanoparticles are synthesized they tend to agglomerate and the latter largely depends on the chemistry as well as the electromagnetic property. This agglomeration also depends on surface energy and thermodynamic instability of the synthesized Ag nanoparticles²⁸. A stabilizing agent depends on the electrostatic repulsion force caused by surface charge, steric stability or both. To prevent their agglomeration the synthesized nanoparticles are coated with the non-magnetic substances in order to maintain their homogeneity. Different types of stabilizing agents have been used²⁹ including PEG (used in the present studies).

SEM images obtained for each sample revealed high density BNP's synthesized from the plant extracts. This analysis displayed individual nanoparticles as well as number of aggregates. Samples with aggregate morphology were ultrasonicated and each nanoparticle was separated.

Table 5—Agar Disc diffusion method using *V. cholerae* against cholerae fever

[Values are mean±SE of 4 replicates]

Sample used	BNP's conc. (ppm)	Result (Zone of inhibition diameter mm)			
		Control			
		Tetra.	Strep.	Strep.	BNP's
<i>T. procumbens</i>					
Fresh leaf	40	29±0.5	39.5±0.5	36±0.8	30±0.8
Fresh stem	40	34±0.8	37±0.5	33±0.5	26

Tetra.=Tetracycline; Strep.=Streptomycin;

BNP's=Biogenic nanoparticles; TNP=Tridax nanoparticles

Table 6—Sample comparisons of plant extracts and TNP's for their antibacterial activity

[Values are mean±SE of 4 replicates]

Sample used	Sample used (zone of Inhibition, mm)				
	AE	EE	ME	TNP,s	SN
<i>E. coli</i>					
<i>T. procumbens</i>					
Fresh leaf	14.5±0.9	11±0.5	NSR	17.5±0.5	28.5±0.9
Fresh stem	9±0.5	7±0.5	8.5±0.5	11.5±0.5	21.5±0.5
<i>V. cholerae</i>					
Fresh leaf	10±0.8	NSR	NSR	27±0.5	35.5±1.2
Fresh stem	NSR	NSR	NSR	29±0.5	37.5±0.9

AE=Aqueous extract; EE=Ethanolic extract; ME=Methanolic extract; TNP=Tridax nanoparticles; SN=Silver nitrate

The XRD pattern of biogenic nanoparticles clearly illustrated that the synthesized nanoparticles were crystalline in nature. In order to verify the results of UV-analysis, the sample of Ag⁺ exposed to plant extracts were examined by XRD. After the reaction

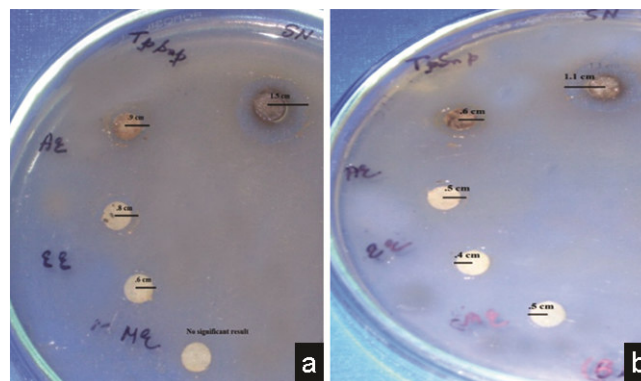


Fig. 10—Results of Disc Diffusion method using leaf (a) and stem (b) derived nanoparticles, when compared with the aqueous (AE), ethanolic (EE) and methanolic (ME) extracts of plant along with silver nitrate (SN)

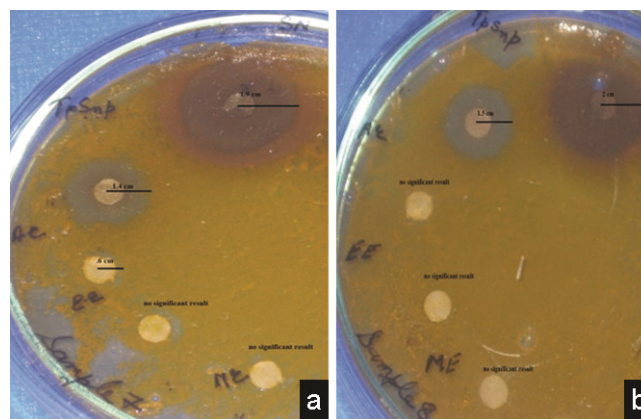


Fig. 11—Showing results of Disc Diffusion method using leaf (a) and stem (b) derived and comparing with the aqueous (AE), ethanolic (EE) and methanolic (ME) extracts of plant, along with silver nitrate (SN).

the diffraction peaks were obtained at 2θ ranging from $0-60^\circ$. Most of the relevant peaks obtained were at 19.3° and 23.4° . Few unassigned peaks were also noticed in the vicinity of the characteristic peaks. These sharp Bragg's peaks might have resulted due to the capping agent stabilizing the nanoparticles³⁰. The line broadening in XRD graph is due to smaller particle size which is in confirmation of a report in *Nicotiana*³¹.

Taken all the available studies together, it is inferred that the size of nanoparticles varied in different species e.g. in *Nicotiana* it was 8 nm³¹ whereas in *Coriandrum* 26 nm³², in *C. quadrangularis* the range was 5-30 nm³³ in comparison to our studies where we encountered nanoparticles ranging from 8.27 to 21.84 nm from both the sources.

Interestingly BNP's synthesized through green route are shown to be inhibitory against pathogenic bacteria *E. coli* and *V. cholerae*. The antimicrobial activity was authenticated through food poisoning and disc diffusion methods. Different concentrations (20, 30, 40, 50 and 60 ppm) of BNP's were used in the present study; of this 30 and 40 ppm were recorded as Minimum inhibitory concentration for *E. coli* and *V. cholerae*, respectively. The present results support the observations of Mahitha *et al*³⁴ who reported the antibacterial effect of silver nanoparticles against the gram negative bacterium (*E.coli* and *K. pneumonia*).

It is generally suggested that the antibacterial effect of Ag mediated nanoparticles is associated with the peptidoglycan layer, though the precise mechanism of its inhibitory effect on microorganisms is not transparent. Some workers have also suggested that the positive charge on the Ag ion is crucial due to the electrostatic attraction between negative charged cell membrane of micro organism and positive charged nanoparticles³⁵. It is also reported that the concentration of Ag nanoparticles affect Gram-negative bacteria through the formation of pits in the cell wall³⁶. Suggestively, there is accumulation of Ag nanoparticles in the bacterial membrane causing alteration in permeability and resulting in cell death. According to Amro *et al.*³⁷ another reason for cell death could be attributed to metal depletion resulting in the formation of irregularly shaped pits in the outer membrane. Consequently, membrane permeability is distorted, due to progressive release of lipopolysaccharide molecules and membrane proteins.

Danilczuk and co-worker³⁸, through the ESR study of Ag nanoparticles, have reported that the

antimicrobial mechanism of Ag nanoparticles is somewhat associated to the formation of free radicals which damage the membrane. Yet another possible mechanism suggested is that the free uptake of free silver ions causes disruption of ATP production and DNA replication³⁹.

It is also reported that synthesized BNP's also exhibit antifungal effects, which is attributed to the destruction of their membrane integrity⁴⁰ and inhibiting the normal budding process by destroying the cell membrane⁴¹.

In conclusion, Ag based nanoparticles prepared by the cost-effective-reduction method described here have great promise as antimicrobial agents. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

Another important experiment was performed for comparing the antimicrobial activity of plant extracts (aqueous, ethanolic and methanolic) and BNP's obtained from them. The intent was to identify the property which could be attributed more to the antimicrobial behaviour of the particles. It was found that while studying the antibacterial activity using *E. coli* and *V. cholerae* BNP's obtained from different plant organs (stem and leaf) showed positive antibacterial activity than any other extracts. Considering the results obtained presently indicated that leaf derived BNP's (fresh extract) showed the best antibacterial activity.

Conclusion

There is a growing need to develop clean and nontoxic procedures for synthesis and assembly of nanoparticles and the extracts obtained from fresh leaf and stem of *T. procumbens* are capable of producing silver nanoparticles extracellular and are quite stable in solution. The rate of reaction for the biosynthesis of these nanoparticles is rapid and ecofriendly, which can be used as an alternative to chemical synthesis protocols at low cost. Change in colour of the reaction mixture during the time of incubation indicated the formation of silver nanoparticles and was confirmed by the characteristic peaks obtained by UV-visible spectra analysis. The size of particles was well characterized by SEM and XRD analysis. It can be predicted that the formation of nanoparticles occurs due to the presence of lactone, ketones, phenols and amines, which are abundantly found in *T. procumbens* and are also confirmed from the FTIR spectra. The

antimicrobial analysis provides strong evidence that the synthesized silver nanoparticles were effective against human pathogens. Metal nanoparticles by herbal approach reported in the present study using *T. procumbens* may have persuasive application in medicine therapeutics and diagnostics.

Acknowledgement

Thanks are due to the management of Rajasthan University-Jaipur, for facility for lyophilisation and management of IIT-Kanpur for helping in characterization of synthesized nanoparticles through SEM, XRD and FTIR analysis.

References

- Cheon J & Horace G, Inorganic nanoparticles for biological sensing, imaging and therapeutics, *Jour Mater Chem*, 19 (2009) 6249.
- Shankar S S, Rai A, Ankamwar B, Singh A, Ahmad A & Sastry M, Biological synthesis of triangular gold nanoprisms, *Nat. Mater*, 3 (2004) 482.
- Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar S R, Khan M I, Parishcha R, Ajaykumar P V, Alam M, Kumar R & Sastry M, Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis, *Nano Lett*, 1 (2001) 515.
- Li S, Shen Y, Xie A, Yu X, Qui L, Zhang L & Zhang Q, Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract, *Green Chem*, 9 (2007) 852.
- Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G & Mukherjee P, The use of microorganisms for synthesis of metal nanoparticles and their application, *Appl Microbiol Biotechnol*, 69 (2006) 485.
- Iravani S, Green synthesis of metal nanoparticles using plants, *Green Chem*, 13 (2011) 2638.
- Nair B & Pradeep T, Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains, *Cryst Growth Des*, 2 (2000) 293.
- Ahmad A, Senapati S, Khan M I, Kumar R & Sastry M, Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora* sp, *Langmuir*, 19 (2003) 3550.
- Ikewuchi C, Jude C, Ikewuchi Catherine M & Igboi Ngozi, Chemical Profile of *Tridax procumbens* Linn. *Pak Nut*, 8(5) (2009) 548.
- Saraf S, Pathak A & Dixit V K, Hair growth promoting activity of *Tridax procumbens*, *Fitoterapia*, 62 (1991) 495.
- Taddei A & Rosas-Romero A J, Bioactivity studies of extracts from *Tridax procumbens*, *Phytomedicine*, 7 (2000) 235.
- Tiwari U, Rastogi B, Singh P, Saraef D K & Vays S P, Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals, *Ethnopharmacol*, 92 (2004) 113.
- Salahdeen H M, Yemitan O K & Alada A R A, Effect of aqueous leaf extract of *Tridax procumbens* on blood pressure and heart rate in rats, *African J Biomed Res*, 7 (2004) 27.
- Ravikumar V, Shivashangari K S & Devaki T, Hepatoprotective activity of *Tridax procumbens* against d-galactosamine/lipopolysaccharide- induced hepatitis in rats, *Ethnopharmacol*, 101 (2005) 55.
- Mulvaney P, Surface plasmon spectroscopy of nanosized metal particles, *Langmuir*, 12 (1996) 788.
- Tolaymat T M E, Badawy A M, Genaidy A, Scheckel K G, Luxton T P & Suidan M, An evidence-based environmental perspective of manufactured silver nanoparticle in synthesis and applications: A systematic review and critical appraisal of peer-reviewed scientific papers, *Sci Total Environ*, 408 (2010) 999.
- Bar H, Bhui D K, Sahoo G P, Sarkar P, Pyne S & Misra A, Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*, *Colloids Surf A Physicochem Eng Asp*, 348 (2009b) 212.
- Bar H, Bhui D H, Sahoo P G, Sarkar P, De P S & Misra A, Green synthesis of silver nanoparticles using latex of *Jatropha curcas*, *Colloids Surf A Physicochem Eng Asp*, 339 (2009a) 134.
- Willner I, Baron R & Willner B, Growing metal nanoparticles by enzymes, *Adv Mater*, 18 (2006) 1109.
- Chandran P S, Chaudhary M, Pasricha R, Ahmad A & Sastry M, Synthesis of gold triangles and silver nanoparticles using *Aloe vera* plant extract, *Biotechnol Prog*, 27 (2006) 577.
- Satyavani K, Ramanathan T & Gurudeeban S, Green synthesis of silver nanoparticles by using stem derived callus extract of bitter apple (*Citrullus colocynthis*), *Digest Jour of Nanomat and Bios*, 6 (2011) 1019.
- Savithramma N, Rao M L & Devi P S, Evaluation of antibacterial efficacy of biologically synthesized silver nanoparticles using bark of *Boswellia ovalifoliolata* Bal. and Henry and Shorea tumbuggaia Roxb, *Biol Sci*, (2011) DOI: 10.3923/jbs.2011.
- Kushwaha H B & Malik C P, Nanofabrication of Silver Nanoparticles from the Stem and Leaf Extract of *Verbesina encelioides*, *Natl Acad Sci Lett*, 35 (2012) 555.
- Kushwaha H B & Malik C P, Biopotential of *Verbesina encelioides* (Stem and Leaf Powder) in Silver Nanoparticles Fabrication, *Turk Biol*, (2013) DOI:10.3906/biy-1212-7
- Kushwaha H B & Malik C P, Biomimetic of silver nanoparticles from dried extracts of *Tridax procumbens* L. (Stem and Leaf), *Plant Sci Res*, 28(2) (2012) 229.
- Dhanalakshmi T & Rajendran S, Synthesis of silver nanoparticles using *Tridax procumbens* and its antimicrobial activity, *Scholars Res Library*, 4 (3) (2012) 1289.
- Umashankari J, Inbakandan D, Ajithkumar T T & Balasub T, Mangrove plant, *Rhizophora mucronata* (Lamk, 1804) mediated one pot green synthesis of silver nanoparticles and its antibacterial activity against aquatic pathogens, *Saline Systems*, 8 (2012) 11.
- Olenin A, Romanovskaya G, Krutyakov Y, Lisichkin G & Zuev V, Sensitized fluorescence of silver nanoparticles in the presence of Pyrene, *Doklady Chem*, 419 (2008) 91.
- Si S & Mandal T K, pH-controlled reversible assembly of peptide-functionalized gold nanoparticles, *Langmuir*, 23(1) (2007) 190.
- Gong P, Li H, He X, Wang K, Hu J, Zhang S & Yang X, Preparation and antibacterial activity of Fe₃O₄ mediated Ag nanoparticles, *Nanotech*, 18(28) (2007) 604.
- Prasad K, Pathak D, Patel A, Dalwadi P, Prasad R, Patel P & Selvaraj Kaliaperumal, Biogenic synthesis of silver nanoparticles using *Nicotiana tabacum* leaf extract and study

- of their antibacterial effect, *Afr Jour Biotechnol*, 10(41) (2011) 8122.
- 32 Sathyavani R, Balamuali K M, Rao S V, Saritha R & Rao N, Biosynthesis of silver nanoparticles using *Corriandrum sativum* leaf extract and their application in nonlinear optics, *Adv Sci Letters*, 3 (2010) 1.
- 33 Renugadevi K, Inbakandan D, Bavanilatha M & Poornima V, *Cissus quadrangularis* assisted biosynthesis of silver nanoparticles with antimicrobial and anticancer potentials, *Int Pharm Bio Sci*, 3(3) (2012) 437.
- 34 Mahitha B, Deva Prasad, Raju B, Dillip G R, Reddy C M, Mallikarjuna K, Manoj L, Priyanka S, Rao K J & Sushma N J, Biosynthesis, characterization and antimicrobial studies of AgNPs extract from *Bacopa monniera* whole plant, *Dig Jour Nanomater Bios*, 6 (2011) 135.
- 35 Dibrov P, Dzioba J, Gosink K K & Hase C C, Chemiosmotic mechanism of antimicrobial activity of Ag in *Vibrio cholera*, *Antimicrob Agents Chemother*, 46 (2002) 2668.
- 36 Soni I & Salopek-Soni B, Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria, *Jour Colloid Interface Sci*, 275 (2004) 177.
- 37 Amro N A, Kotra L P, Wadu-Mesthrige K, Bulychiev A, Mobashery S & Liu G, High-resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: structural basis for permeability, *Langmuir*, 16 (2000) 2789.
- 38 Danilczuk M, Lund A, Saldo J, Yamada H & Michalik J, Conduction electron spin resonance of small silver particles, *Spectrochim Acta A*, 63 (2006) 189.
- 39 Lok C N, Ho C M, Chen R, He Q Y, Yu W Y, Sun H, Tam P K, Chiu J F & Che C M, Proteomic analysis of the mode of antibacterial action of silver nanoparticles, *Proteome Res*, 5 (2006) 916.
- 40 Kim K J, Sung W S, Suh B K, Seok K M, Jung S C, Jung G K & Dong G L, Antifungal activity and mode of action of silver nano-particles on *Candida albicans*, *Biometals*, 22(2) (2009) 235.
- 41 Praveen A, Roy A S & Rao S, Biosynthesis and characterization of silver nanoparticles from *Cassia auriculata* leaf extract and *in vitro* evaluation of antimicrobial activity, *Inter Appl Biol Pharm Tech*, 3(2) (2012) 222.